CDK inhibition and cancer therapy

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The cell-division cycle is a tightly controlled process that is regulated by the cyclin/CDK family of protein kinase complexes. Stringent control of this process is essential to ensure that DNA synthesis and subsequent mitotic division are accurately and coordinately executed. There is now strong evidence that CDKs, their regulators, and substrates are the targets of genetic alteration in many human cancers. As a result of this, the CDKs have been targeted for drug discovery and a number of small molecule inhibitors of CDKs have been identified.

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Abbreviations

CDK	cyclin-dependent kinase
CKI	CDK inhibitory protein
DMAP	6-dimethyl aminopurine
EGF	epidermal growth factor
NGF	nerve growth factor
RB	retinoblastoma

Introduction

Mammalian cell division is regulated by the timely and coordinated activation of the cyclin-dependent kinase (CDK) family. Regulation of CDK activity occurs at multiple levels, including cyclin synthesis and degradation; activating and inactivating phosphorylation events; CDK inhibitor protein synthesis, binding and degradation; and subcellular localization ([1–5]; see Figure 1). Undoubtedly with the magnitude of research directed at CDKs, further insights into novel mechanisms of their regulation will be revealed. Regulation of CDK activity is essential to the ordered execution of the processes that govern cell growth, complete DNA replication and mitotic transfer of the genome to new daughter cells. To ensure this, surveillance mechanisms function as checkpoints to control cell-cycle progression in case the conditions for advancement have not been met [6,8,9•,10]. As one of their functions, these signaling pathways exert their effects on cell-cycle progression through CDK regulation. Similarly, as part of their function, growth-promoting signal transduction pathways must transmit their effects on cell-cycle progression by modulating CDK activity [11–14]. As with components of these signal transduction pathways that are so often genetically altered in human cancers, it is befitting that CDKs, their regulators, and substrates, are also frequently the targets of genetic lesions, and promote neoplastic transformation [15,16]. The best-characterized case of such alteration is the retinoblastoma (RB) pathway. Under normal conditions, phosphorylation of pRb by the Cdk4 or Cdk6 enzyme in complex with one of the D-type cyclins are required for $G_1 \rightarrow S$ phase transition. Conversely, pRb's unphosphorylated state is essential for mitotic division cycle exit. Cdk4 and Cdk6 are specifically inhibited by the INK4 small molecular weight CDK inhibitor family. It is noted that alterations in one or another component of this pathway is found in nearly all human cancers [15–17].

Excellent reviews have recently documented the multiple modes of CDK regulation, interactions between CDK regulatory pathways and checkpoint control mechanisms and oncogenic alterations of cell-cycle components. Our attempt here is to illustrate the potential for development of therapeutics to treat human cancers by interfering with cell-cycle progression. Because of the central role that they play in advancing the division cycle, CDKs have been targeted for drug discovery and a number of small molecule compounds have now been identified as CDK inhibitors. These strategies and other targets of intervention within the cell cycle are discussed in our review.

Approaches to CDK inhibition

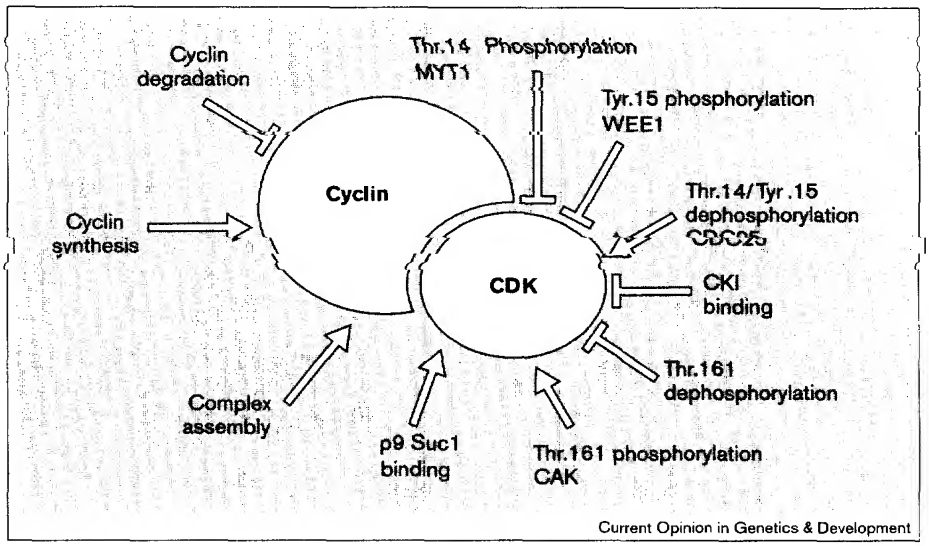
Because of the complex nature of its regulation, modulating CDK activity can be approached via multiple modes for therapeutic intervention. Two basic schemes to inhibit cyclin-dependent kinases are to either directly block the catalytic activity of the CDK, or to target the major regulators of their activity. The most extensively examined of these is catalytic inhibition, which has produced both chemical and peptide/protein based CDK inhibitors. Regulators of CDK activity amenable to therapeutics can encompass: factors involved in the expression and synthesis of the CDK/cyclin subunits or CDK inhibitory proteins, CKIs; proteins that regulate the phosphorylation state of CDKs such as CAK, Cdc25 phosphatases, and the Wee1 and Myt1 kinases; and the machinery involved in proteolytic degradation of the CDK/cyclin complexes or their regulators.

Chemical inhibitors of CDKs

There are six classes of CDK inhibitors that have thus far been characterized: the purine-based compound olomoucine and its analogues, butyrolactone, flavopiridol, staurosporine and the related compound UCN-01, suramin and 9-hydroxyellipticine. Each is either a natural product or derivative of one with a distinct chemical structure. All occupy the ATP-binding pocket of the enzyme and are competitive with ATP. When examining inhibitors that bind to the catalytic site, especially the catalytic site of an enzyme belonging to a large family such as kinases, the issue of specificity becomes a major issue. However, recent experience and success with the development of effective and specific ATP-competitive inhibitors of a number of

Figure 1

Multiple modes of CDK regulation. Regulation of CDK activity occurs at multiple levels, as outlined here. (Thr, threonine; Tyr, tyrosine.) The cdc2 enzyme is used as a reference for sites of phosphorylation (i.e Thr.14, Tyr.15 and Thr.161). With regard to phosphorylation, the name of the enzyme responsible for a phosphorylation event is given below the event decribed, for example, threonine 14 phosphorylation is carried out by the MYT1 enzyme.



kinase enzymes has shown that this task is achievable. Olomoucine and its analogues, butyrolactone, and flavopiridol all show strong specificity for CDKs versus a number of unrelated kinases (see Figure 2 for their chemical structures). Staurosporine, UCN-01 and suramin, on the other hand, show no specificity between the CDKs and other kinases such as PKC [18]. In some cases, such as for 9-hydroxyellipticine the inhibitory activity against kinases other than Cdk2 and Cdc2 is unknown [19,20]. It is interesting to note that of these compounds both olomoucine and butyrolactone inhibit Cdc2 and closely related kinases but do not affect the cyclin-D-dependent kinases Cdk4 and Cdk6. Flavopiridol, on the other hand, can inhibit all CDKs tested including Cdk4 [18]. In collaboration with Parke-Davis Pharmaceutical Research, we have recently identified a chemical inhibitor of the Cdk4 and Cdk6 enzymes by high-throughput screening of a large compound library. This ATP-competitive inhibitor is the first to demonstrate great specificity towards these enzymes versus other CDKs and unrelated kinases (MD Garrett, A Fattaey, unpublished data). In the interests of space, we only discuss further the three classes of CDK inhibitors that show strong specificity for CDKs versus other kinases.

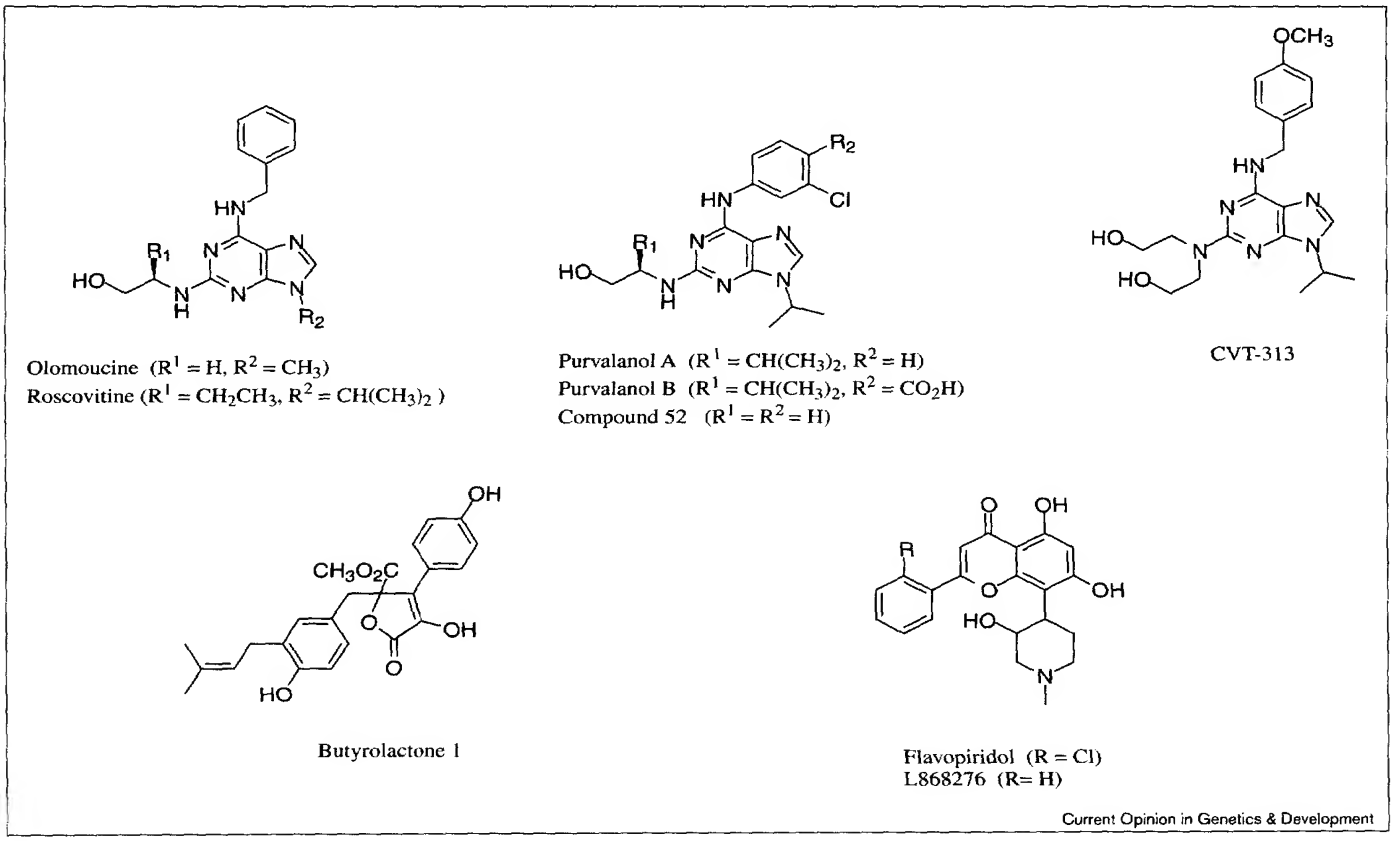
Olomoucine, roscovitine, CVT-313 and purvalanol

The first compound identified as a Cdc2 inhibitor was 6-dimethyl aminopurine (DMAP; IC50 = 120 μM). DMAP was originally synthesized as a puromycin analogue that blocked mitosis in sea urchin embryos without inhibiting protein synthesis [21–23]. Structural analogy searches identified isopentyl adenine as a slightly more potent DMAP analogue with an IC50 of 55 μM [24]. This strategy lead to the discovery of olomoucine as a potent Cdc2 inhibitor (IC50 = 7 μM) with specificity for a subset of CDKs [25]. Among 35 kinases tested, olomoucine only inhibited Cdc2, Cdk2, Cdk5 and MAP kinase in the micromolar range and did not affect Cdk4 or Cdk6 [25]. Crystallization of olomoucine and a related but weaker inhibitor, isopentyl adenine, with Cdk2 revealed that, although both bound in the ATP-binding pocket of the CDK, the adenine side-chain of each lay in a completely different orientation from the adenine group of ATP [26]. Furthermore, the N6 substituent of olomoucine bound outside the conserved region of the binding pocket making contacts with the protein that are not possible for ATP, suggesting that this interaction is most likely responsible for the specificity of olomoucine towards CDKs.

Another substituted purine compound, roscovitine, is a 10-fold more potent Cdc2 inhibitor compared to olomoucine [27,28•]. As evident from its crystal structure with Cdk2, roscovitine binds in a similar orientation as olomoucine, with the N6 substituent also making contacts outside the conserved binding domain affirming the likelihood that this interaction provides the specificity of this compound class for CDKs [28•]. A related compound, CVT-313, has similar inhibitory activity against CDKs as roscovitine and will block neointimal formation in a rat carotid artery restenosis model [29•].

The most recent addition to these purine-based structures is a group of compounds that were identified in a screen of trisubstituted purine combinatorial libraries designed for Cdk2 inhibition [30••]. The best of these *in vitro* is purvalanol B which shows a thousand-fold increase in potency against Cdk2/Cyclin A when compared to olomoucine. Less potent but more membrane-permeable are purvalanol A (IC50 Cdk2/cyclin A = 70 nM) and compound 52. Crystallographic analysis of purvalanol B with Cdk2 reveals that binding into the ATP-binding pocket resembles that of the other substituted purines olomoucine and

Figure 2



Chemical inhibitors of CDKs. The structures shown in this figure are of those compounds which specifically inhibit the CDK family of kinases. It should be noted that olomoucine, roscovitine, purvalanol A, purvalanol B, compound 52 and CVT-313 are all trisubstituted purines and thus structurally related.

roscovitine, including the orientation of the N6 substituent away from the conserved region of the pocket, providing specificity towards the CDKs [30••].

Both olomoucine and roscovitine can arrest a number of different cell types in the G_1 and G_2 phases of the cell cycle and block known CDK-dependent cellular activities [31,32•]. For olomoucine, these include phosphorylation of the Cdc2 substrates protein phosphatase 1 and elongation factors α and γ, and disassembly of the mitotic Golgi apparatus, whereas roscovitine will block phosphorylation of the Cdc2/cyclin B substrate vimentin [31,32•,33,34]. In a similar fashion, CVT-313 can block phosphorylation of pRb, a known CDK substrate, and arrest progression through the cell cycle at the G_1/S and G_2/M boundaries [29•]. No cell-cycle data is available for purvalanol A or B. Comparison of the complete mRNA expression profile analysis of a yeast cdc28 mutant with that of wild-type cells treated with compound 52 or flavopiridol revealed the modulation of a common set of genes' expression, suggesting that in this system, both drugs may be inhibiting the cdc28 kinase. Taken together, these data provide sufficient evidence that this class of compounds can inhibit multiple CDKs in the cell. There are also data now emerging that at least olomoucine and roscovitine can inhibit the growth of cancer cell lines [31,32•,35,36].

Butyrolactone

Butyrolactone was originally identified as a new type of metabolite from *Aspergillus terreus* var. *africanus* IFO8835. It was independently re-isolated from cultured medium of micro-organisms screened for inhibitors of Cdc2/cyclinB [37]. As with the DMAP-derived class of compounds, butyrolactone can inhibit Cdc2 and Cdk2, but not Cdk4, and blocks pRb phosphorylation and entry into S phase in the human WI-38 cell line [18,38]. Butyrolactone also blocks histone phosphorylation and progression through G_2/M in synchronized WI-38 cells. Cellular effects of butyrolactone can be augmented if cells are treated with digitonin, indicating its poor permeability [39]. Its antitumor effects against several lung cancer cell lines *in vitro*, however, have now been well documented [39–41].

Flavopiridol (L86-8275)

Flavopiridol is a synthetic analogue of a natural alkaloid isolated from the stem bark of *Dysoxylum binectariferum*, a plant found in India [42]. It was first identified in a tandem screening of the EGF receptor tyrosine kinase inhibition

(IC50 = 21 μM) combined with analysis of inherent cellular cytotoxicity but it was shown to have a far more potent activity against a number of CDKs, including Cdk4. The crystal structure of the related compound L86–8276 (deschloro-flavopiridol) with Cdk2 has revealed that whereas the aromatic portion of the inhibitor binds into the ATP-binding pocket of Cdk2, the phenyl group makes contacts outside of the pocket [43]. This latter association is in a similar manner to the N6 substituent on olomoucine, roscovitine and purvalanol B. This result further strengthens the concept that association of part of a chemical structure with this region of the Cdk2 molecule is important for its specificity towards the CDKs.

As with the purine-based CDK inhibitors, flavopiridol can produce a block in cell-cycle progression at G_1 and G_2, when added to an asynchronous population of cells [42]. In the breast carcinoma line MCF7, addition of flavopiridol leads to loss of pRb phosphorylation and G_1 arrest [44]. Surprisingly, in these studies, Cdk4 activity is elevated up to three hours after compound addition but has completely disappeared 12 hours post treatment. This lack of kinase activity correlates with the disappearance of cyclin D suggesting that inhibition may be caused by removal of the cyclin partner and not to direct inhibition of the catalytic activity of the enzyme. Flavopiridol has clearly been shown to have growth-inhibitory activity against a number of tumor types *in vitro* and *in vivo* [45–48]. Flavopiridol is now in clinical trials as an anti-cancer drug [49,50].

CDK inhibitory proteins

Since their discovery, CKIs have frequently been used to investigate the effects of CDK inhibition on tumor-cell growth. Combined with adenoviral vectors as a vehicle for delivery and expression, this is a powerful approach for examining therapeutic applications of CDK inhibition. Adenoviral-mediated expression of CKIs was first described in a report showing that introduction of p16 into lung cancer lines either deleted or, with no detectable expression of the gene, blocked entry into S phase and caused growth inhibition [51]. Tumor studies using these virally infected lines *in vivo* also displayed growth inhibition when p16 was expressed. Since then, a number of similar studies in a variety of tumor types have also demonstrated growth inhibition both *in vitro* and *in vivo*.

Analogous to transfection studies carried out with p16, adenovirus-mediated p16 expression causes growth inhibition in cells with a functional RB gene product but not in tumor lines where it is deleted or mutated [52–55]. Interestingly, none of these studies reported significant apoptosis upon p16 expression; however, co-infection of p16- and p53-expressing adenoviruses could induce significant apoptosis in tumor lines [56]. Studies with adenoviruses expressing either p21 or p27 in cancer cell lines have also demonstrated both *in vitro* and *in vivo* growth inhibition [53,57–63]. Interestingly, two studies with a recombinant adenovirus expressing p27 have reported significant apoptosis in a number of cancer lines *in vitro* [64,65] but it is not proven that this effect is caused by CDK inhibition. In conclusion, gene therapy for cancer using adenoviruses to express p16, p21 or p27 has provided promising results in preclinical studies and could be the basis of a new strategy for cancer gene therapy.

Peptide-based inhibitors of CDK activity

Peptides that mimic the CDK-inhibitory activity of either p16 or p21 have proven useful as a tool in understanding the changes in cell growth and phenotype caused by these inhibitors. This novel approach combines the power of CDK inhibition with the transmembrane carrier function of a 16 amino acid region of the *Drosophila* Antennapedia protein to deliver the inhibitor into live cells. Treatment of cells with a hybrid peptide corresponding to the third ankyrin repeat of p16, which can bind to and inhibit both Cdk4 and Cdk6 fused to the Antennapedia carrier sequence induced an RB-dependent G_1 cell-cycle arrest and cellular characteristics associated with senescence [66,67,68••]. Full-length recombinant p16 attached to the same 16 amino acid Antennapedia-carrier sequence had similar cellular effects suggesting that, as with adenovirus mediated p16 expression, reconstitution of p16-mediated Cdk4/6 inhibitory activity in cancer cells causes growth inhibition and senescence [69].

Synthetic peptides corresponding to the p21 protein sequence have also been generated but with differing cellular effects. One peptide corresponding to the carboxy-terminal region of p21 (amino acids 141–160) had a 40-fold higher specificity for Cdk4/cyclinD versus Cdk2/cyclinE and gave a potent G_1 arrest in breast cancer lines [70]. Two other p21 peptides generated against amino-terminal regions of p21 (amino acids 17–33 and 63–77) inhibited both Cdk2 and Cdc2 *in vitro* and gave a general blockade in all phases of the cell cycle when introduced into cells [71]. Finally, a number of 20 amino acid peptide aptamers have been identified from a combinatorial library that bind to and inhibit Cdk2 kinase activity in the nanomolar range *in vitro*. At least one of these aptamers appears to do this by blocking the interaction of Cdk2 with its protein partners or substrates [72]. In the future, this technology could be used to identify aptamers that specifically inhibit a variety of protein targets. These approaches suggest that peptidomimetics of CKIs or peptides that inhibit CDK activity could provide novel templates for the development of anti-cancer drugs.

CDK inhibition: growth arrest, senescence and apoptosis

There are a number of ways to study the role of CDKs in the cell, the simplest being to assess the cellular response to CDK inhibition. In yeast, the power of genetics and the regulation of cell cycle by a single CDK enzyme has been invaluable in understanding the consequences of loss of CDK activity. In human cells, however, the complexity of the CDK family and the programmed suicide system (apoptosis) triggered in response to growth and cell-cycle

perturbations, combined with the multitude of the different cell types that the studies are conducted in, makes the task of understanding the consequences of CDK inhibition rather complicated and confusing. Nonetheless, important knowledge can be gained to understand the implications of CDK inhibition for therapeutic purposes. Cell-cycle arrest followed by either cell differentiation or induction of apoptosis are visibly the most common phenotypes encountered upon inhibition of CDKs in human cells. Van den Heuvel and Harlow [73] first described the use of dominant negative forms of CDKs (DN-CDKs) which showed that overexpression of DN-Cdc2 alleles could specifically arrest cells at the G_2/M transition whereas expression of DN-Cdk2 and Cdk3 resulted in G_1 arrest [73]. It has been shown more recently that DN-Cdc2, Cdk2 and Cdk3 will suppress apoptosis in HeLa cells induced by staurosporine and TNFα whereas DN-Cdk2 but not DN-Cdc2 will block apoptosis induced by forced overexpression of topoisomerase II α [74,75]. In contrast, DN-Cdk4 and Cdk6 but not DN-Cdk2 or Cdk3 will protect cultured neurons against DNA damage or NGF deprivation induced cell death, suggesting that different CDKs may play a role in apoptosis depending on the cell type [76]. Heterologous expression of p16 and p21 or peptidomimetics derived from these CKIs also induce G_1 arrest and, in some cases, a senescent phenotype but no apparent apoptosis (see previous two sections). There have been two reports, however, suggesting that overexpression of p27 leads to apoptosis [64,65].

The chemical inhibitors olomoucine, roscovitine, butyrolactone and flavopiridol all arrest proliferating cells at the G_1/S and G_2/M boundaries and induce apoptosis [31,32•,40,41,77–79]. Olomoucine will also trigger an apoptotic response in cells that have been arrested in G_2 by DNA-damaging agents, whereas flavopiridol can induce apoptosis in non-cycling cells [48,80]. In contrast, all these chemical inhibitors can block apoptosis in neuronal cells [81–83]. Finally, there have been recent reports that cleavage of CKIs p21 and/or p27 by caspases leads to Cdk2 activation when apoptosis is induced in human cells [84–86]. In one case, this phenotype could be partially suppressed by expression of DN-Cdk2. Fas-induced apoptosis in jurkat cells has been reported to cause cleavage of the CDK inhibitory kinase Wee1 and the Cdc27 protein which is involved in cyclin degradation, providing other routes to CDK activation during apoptosis [87]. This would lead us to the conclusion that there must be several factors that determine whether CDK inhibition leads to induction of apoptosis such as the mechanism of inhibition, the cell type, and whether the cell is in cycle and (in some cases) activation of CDK activity may be part of the apoptotic process itself.

Summing up and the future for CDK inhibitors

We have outlined the potential therapeutic value of CDK inhibition as a cancer treatment and presented the approaches taken to investigate this possibility. To date, only the chemical inhibitor flavopiridol has reached clinical trials and it has yet to be proven whether its effects are caused by CDK inhibition. Specificity of these compounds is a key factor and one of the future challenges will be to generate more potent and specific CDK inhibitors. Further crystallographic studies of CDKs with novel chemical inhibitors should provide more insight into this issue. In the meantime, there are a number of pharmaceutical companies with ongoing CDK inhibitor programs and non-chemical approaches to CDK inhibition are being tested that may provide us with a CDK inhibitor as an anti-cancer drug in the not too distant future.

Future prospects

In line with the prevailing CDK-centric view of the cell-division cycle is the fact that the majority of therapeutics under development that target cell-cycle regulation have thus far been CDK inhibitors. Modulators of CDK activity, however, offer as good or greater potential as targets for intervention. As discussed earlier, a number of CDK-activating enzymes (such as CAK and Cdc25 phosphatases) and inhibitory enzymes (such as Wee1 and Myt1) as well as enzymes involved in modulating their activity, can now be considered.

Although the CAK-dependent CDK activation step appears to be constitutive, inhibitory phosphorylation of CDKs and their activation by Cdc25 phosphatases is highly regulated and is the target of checkpoint surveillance mechanisms [88–90]. This is especially well characterized for the G_2 to M transition and the checkpoint signaling pathway invoked in response to DNA damage where the inhibitory phosphorylation on Cdc2, along with a loss of Cdc2 activity is sustained. Using such agents as caffeine and UCN-01, abrogation of this DNA-damage-induced G_2 checkpoint correlates well with loss of phosphorylation on at least tyrosine 15 of Cdc2 and an increase in Cdc2 activity [91–94]. In addition, exogenous expression of Cdc2 alleles containing non-phosphorylatable amino acids at position 14 and 15 prevents the G_2 arrest induced by ionizing radiation [95–97]. These studies illustrate a significant loss in cell viability, indicating that abrogation of the G_2 checkpoint, especially in p53 null cancer cells, may be a way to target them for cell death.

Another area of CDK/cyclin regulation with therapeutic potential is the control of CDK, cyclin or CKIs levels in the cell. Antisense technologies have recently been applied to eliminate Cdk2 mRNA levels in cells [98–101]. Cyclins and CKIs are regulated at the transcriptional and protein stability level. Much has been learned about the enzyme complexes and the signals involved in targeting the cyclins and to a lesser extent the CKIs for ubiquitin-mediated proteolysis [102,103]. An increase in the proteolysis of specific cyclins may be one way to inactivate the CDKs and conversely inhibition of CKI turnover may yield cell-cycle arrest and cytostasis [104]. The p27 CDK inhibitor may be an attractive choice in this respect, as it is active in mid to late G_1, and its levels are either reduced or absent in a number of malignancies [105–109]. Decreased levels of

p27 are also indicative of poor prognosis in young breast cancer patients [110]. Most recently the murine p27Kip1 gene has been shown to be haploinsufficient for tumor suppression, which establishes a causal link between loss of p27 and a predisposition to tumors [111]. It can therefore be safely said that as our understanding of the cell cycle and its role in tumor progression expands, new approaches and targets for intervention and treatment of human cancers must surely be just around the corner.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Morgan DO, Fisher RP, Espinoza FH, Farrell A, Nourse J, Chamberlin H, Jin P: **Control of eukaryotic cell cycle progression by phosphorylation of cyclin-dependent kinases.** *Cancer J Sci Am* 1998, **4(Suppl 1)**:S77-83.
2. Arellano M, Moreno S: **Regulation of CDK/cyclin complexes during the cell cycle.** *Int J Biochem Cell Biol* 1997, **29**:559-573.
3. Lees E: **Cyclin dependent kinase regulation.** *Curr Opin Cell Biol* 1995, **7**:773-780.
4. Morgan DO: **Principles of CDK regulation.** *Nature* 1995, **374**:131-134.
5. Norbury C, Nurse P: **Animal cell cycles and their control.** *Annu Rev Biochem* 1992, **61**:441-470.
6. Elledge SJ: **Cell cycle checkpoints: preventing an identity crisis.** *Science* 1996, **274**:1664-1672.
7. Nasmyth K: **Viewpoint: putting the cell cycle in order.** *Science* 1996, **274**:1643-1645.
8. Nasmyth K: **At the heart of the budding yeast cell cycle.** *Trends Genet* 1996, **12**:405-412.
9. • Nurse P, Masui Y, Hartwell L: **Understanding the cell cycle.** *Nat Med* 1998, **4**:1103-1106.

A good recent summary (with a historical perspective) on cell-cycle research

10. Paulovich AG, Toczyski DP, Hartwell LH: **When checkpoints fail.** *Cell* 1997, **88**:315-321.
11. Peeper DS, Bernards R: **Communication between the extracellular environment, cytoplasmic signalling cascades and the nuclear cell-cycle machinery.** *FEBS Lett* 1997, **410**:11-16.
12. Peeper DS, Upton TM, Ladha MH, Neuman E, Zalvide J, Bernards R, DeCaprio JA, Ewen MG: **Ras signalling linked to the cell-cycle machinery by the retinoblastoma protein.** *Nature* 1997, **386**:177-181. [published erratum appears in *Nature* 1997 **386**:521.]
13. Lavoie JN, Rivard N, L'Allemain G, Pouyssegur J: **A temporal and biochemical link between growth factor-activated MAP kinases, cyclin D1 induction and cell cycle entry.** *Prog Cell Cycle Res* 1996, **2**:49-58.
14. Aktas H, Cai H, Cooper GM: **Ras links growth factor signaling to the cell cycle machinery via regulation of cyclin D1 and the Cdk inhibitor p27KIP1.** *Mol Cell Biol* 1997, **17**:3850-3857.
15. Hall M, Peters G: **Genetic alterations of cyclins, cyclin-dependent kinases, and Cdk inhibitors in human cancer.** *Adv Cancer Res* 1996, **68**:67-108.
16. Sherr CJ: **Cancer cell cycles.** *Science* 1996, **274**:1672-1677.
17. Weinberg RA: **How cancer arises.** *Sci Am* 1996, **275**:62-70.
18. Meijer L: **Chemical inhibitors of cyclin-dependent kinases.** *Trends Cell Biol* 1996, **6**:393-397.
19. Ohashi M, Sugikawa E, Nakanishi N: **Inhibition of p53 protein phosphorylation by 9-hydroxyellipticine: a possible anticancer mechanism.** *Jpn J Cancer Res* 1995, **86**:819-827.
20. Nomoto T, Nishio K, Saijo N: **Cell cycle regulation by anticancer agent.** *Gan To Kagaku Ryoho* 1995, **22**:1719-1723.
21. Rebhun LI, White D, Sander G, Ivy N: **Cleavage inhibition in marine eggs by puromycin and 6-dimethylaminopurine.** *Exp Cell Res* 1973, **77**:312-318.
22. Neant I, Guerrier P: **6-Dimethylaminopurine blocks starfish oocyte maturation by inhibiting a relevant protein kinase activity.** *Exp Cell Res* 1988, **176**:68-79.
23. Meijer L, Pondaven P: **Cyclic activation of histone H1 kinase during sea urchin egg mitotic divisions.** *Exp Cell Res* 1988, **174**:116-129.
24. Rialet V, Meijer L: **A new screening test for antimitotic compounds using the universal M phase-specific protein kinase, p34cdc2/cyclin Bcdc13, affinity-immobilized on p13suc1-coated microtitration plates.** *Anticancer Res* 1991, **11**:1581-1590.
25. Vesely J, Havlicek L, Strnad M, Blow JJ, Donella-Deana A, Pinna L, Letham DS, Kato J, Detivaud L, Leclerc J: **Inhibition of cyclin-dependent kinases by purine analogues.** *Eur J Biochem* 1994, **224**:771-786.
26. Schulze-Gahmen U, Brandsen J, Jones HD, Morgan DO, Meijer L, Vesely J, Kim SH: **Multiple modes of ligand recognition: crystal structures of cyclin-dependent protein kinase 2 in complex with ATP and two inhibitors, olomoucine and isopentenyladenine.** *Proteins* 1995, **22**:378-391.
27. Havlicek L, Hanus J, Vesley J, Leclerc S, Meijer L, Shaw G, Strnad M: **Cytokinin-derived cyclin-dependent kinase inhibitors: synthesis and cdc2 inhibitory activity of olomoucine and related compounds.** *J Med Chem* 1997, **40**:408-412.
28. • De Azevedo WF, Lecelrc S, Meijer L, Strnad M, Kim SH: **Inhibition of cyclin-dependent kinases by purine analogues: crystal structure of human cdk2 complexed with roscovitine.** *Eur J Biochem* 1997, **243**:518-526.

The introduction of this report gives an excellent review on inhibition of CDKs by purine-based analogues, followed by biochemical and crystallographic information on roscovitine, one of the more recent members of this family.

29. • Brooks EE, Gray NS, Joly A, Kerwar SS, Lum R, Mackman RL, Norman TC, Rosete J, Rowe M, Schow SR *et al.*: **CVT-313, a specific and potent inhibitor of CDK2 that prevents neointimal proliferation.** *J Biol Chem* 1997, **272**:29207-29211.

This reference provides information on the biochemical and cellular effects of CVT-313, one of the newest purine analogues.

30. •• Gray NS, Wodicka L, Thunnisen AM, Norman TC, Kwon S, Espinoza FH, Morgan DO, Barnes G, Leclerc S, Meijer L *et al.*: **Exploiting chemical libraries, structure, and genomics in the search for kinase inhibitors.** *Science* 1998, **281**:533-538.

This is the most recent paper on the purine-based class of CDK inhibitors and is interesting in that it describes how chemical libraries, crystallographic data and genomics can be used to identify more potent CDK inhibitors such as purvalanol A and B.

31. Abraham RT, Acquarone M, Andersen A, Aseni A, Belle R, Berger F, Bergounioux C, Brunn G, Buquet-Fagot C, Fagot D: **Cellular effects of olomoucine, an inhibitor of cyclin-dependent kinases.** *Biol Cell* 1995, **83**:105-120.
32. • Meijer L, Borgne A, Mulner O, Chong JP, Blow JJ, Inagaki N, Inagaki M, Delcros JG, Moulinoux JP: **Biochemical and cellular effects of roscovitine, a potent and selective inhibitor of the cyclin-dependent kinases cdc2, cdk2 and cdk5.** *Eur J Biochem* 1997, **243**:527-536.

This paper was published adjacently to [28•] and reports the biochemical and cellular effects of the purine analogue roscovitine.

33. Misteli T, Warren G: **Mitotic disassembly of the Golgi apparatus *in vivo*.** *J Cell Sci* 1995, **108**:2715-2727.
34. Kwon YG, Lee SY, Choi Y, Greengard P, Nairn AC: **Cell cycle-dependent phosphorylation of mammalian protein phosphatase 1 by cdc2 kinase.** *Proc Natl Acad Sci USA* 1997, **94**:2168-2173.
35. Hajduch M, Kolar Z, Novotny R, Hanus J, Mihal V, Hlobilkova A, Strnad M: **Induction of apoptosis and regression of spontaneous dog melanoma following *in vivo* application of synthetic cyclin-dependent kinase inhibitor olomoucine.** *Anticancer Drugs* 1997, **8**:1007-1013.
36. Iseki H, Ko TC, Xue XY, Seapan A, Hellmich MR, Townsend CM Jr: **Cyclin-dependent kinase inhibitors block proliferation of human gastric cancer cells.** *Surgery* 1997, **122**:187-194; discussion 194-195.
37. Kitagawa M, Okabe T Ogino H, Matsumoto H, Suzucki-Takahashi I, Kokubo T, Higashi H, Taya Y, Yasuda H: **Butyrolactone I, a selective inhibitor of cdk2 and cdc2 kinase.** *Oncogene* 1993, **8**:2425-2432.

38. Kitagawa M, Higashi H, Takahashi IS, Okabe T, Ogino H, Taya Y, Hisumura S, Okuyama A: **A cyclin-dependent kinase inhibitor, butyrolactone I, inhibits phosphorylation of RB protein and cell cycle progression.** *Oncogene* 1994, **9**:2549-2557.

39. Nishio K, Ishida T, Arioka H, Kurokawa H, Fukuoka K, Nomoto T, Fukumoto H, Yokote H, Saijon N: **Antitumor effects of butyrolactone I, a selective cdc2 kinase inhibitor, on human lung cancer cell lines.** *Anticancer Res* 1996, **16**:3387-3395.

40. Wada M, Hosotani R, Lee JU, Doi R, Koshiba T, Fujimoto K, Miyamoto Y, Tsuji S, Nakajima S, Okuyama A, Imamura M: **An exogenous cdk inhibitor, butyrolactone-I, induces apoptosis with increased Bax/Bcl-2 ratio in p53-mutated pancreatic cancer cells.** *Anticancer Res* 1998, **18**:2559-2566.

41. Yamamoto H, Monden T, Miyoshi H, Izawa H, Ikeda K, Tsujie M, Ohnishi T, Sekimoto M, Tomita N, Monden M *et al.*: **Cdk2/cdc2 expression in colon carcinogenesis and effects of cdk2/cdc2 inhibitor in colon cancer cells.** *Int J Oncol* 1998, **13**:233-239.

42. Hans Harald Sedlacek JC, Naik R, Kaur G, Worland P, Losiewicz M, Parker B, Carlson B, Smith A, Senderowicz A, Sausville E: **Glavopiridol (L86 8275; NSC 649890), a new kinase inhibitor for tumor therapy.** *Int J Oncol* 1996, **9**:1143-1168.

43. De Azevedo WF Jr, Muller-Dieckmann HJ, Schulze-Gahmen U, Worland PJ, Sausville E, Kim SH : **Structural basis for specificity and potency of a flavonoid inhibitor of human CDK2, a cell cycle kinase.** *Proc Natl Acad Sci USA* 1996, **93**:2735-2740.

44. Carlson BA, Dubay MM, Sausville EA, Brizuela L, Worland PJ: **Flavopiridol induces G1 arrest with inhibition of cyclin-dependent kinase (CDK) 2 and CDK4 in human breast carcinoma cells.** *Cancer Res* 1996, **56**:2973-2978.

45. Patel V, Senderowicz AM, Pinto D, Igishi T, Raffeld M, Quintailla-Martinez L, Ensley JF, Sausville EA, Silvio Gutkind J: **Flavopiridol, a novel cyclin-dependent kinase inhibitor, suppresses the growth of head and neck squamous cell carcinomas by inducing apoptosis.** *J Clin Invest* 1998, **102**:1674-1681.

46. Kaur G, Stetler-Stevenson M, Sebers S, Worland P, Sedlacek H, Myers C, Czech J, Naik R, Sausville EA: **Growth inhibition with reversible cell cycle arrest of carcinoma cells by flavone L86-8275.** *J Natl Cancer Inst* 1992, **84**:1736-1740.

47. Drees M, Dengler WA, Roth T, Labonte H, Mayo J, Malspeis L, Grever M, Sausville EA, Fiebg HH: **Flavopiridol (L86-8275): selective antitumor activity *in vitro* and activity *in vivo* for prostate carcinoma cells.** *Clin Cancer Res* 1997, **3**:273-279.

48. Bible KC, Kaufmann SH: **Flavopiridol: a cytotoxic flavone that induces cell death in noncycling A549 human lung carcinoma cells.** *Cancer Res* 1996, **56**:4856-4861.

49. Wright J, Blatner GL, Cheson BD: **Clinical trials referral resource. Clinical trials of flavopiridol.** *Oncology* 1998, **12**:1018,1023-1014.

50. Senderowicz AM, Headlee D, Stinson SF, Lush RM, Kalil N, Villalba L, Hill K, Steinberg SM, Figg WD, Tompkins A, Arbuck SG, Sausville EA: **Phase I trial of continuous infusion flavopiridol, a novel cyclin-dependent kinase inhibitor, in patients with refractory neoplasms.** *J Clin Oncol* 1998, **16**:2986-2999.

51. Jin X, Nguyen D, Zhang WW, Kyritsis AP, Roth JA: **Cell cycle arrest and inhibition of tumor cell proliferation by the p16INK4 gene mediated by an adenovirus vector.** *Cancer Res* 1995, **55**:3250-3253.

52. Chintala SK, Fueyo J, Gomez-Manzano C, Venkaiah B, Bjerkvig R, Yung WK, Sawaya R, Kyritsis AP, Rao JS: **Adenovirus-mediated p16/CDKN2 gene transfer suppresses glioma invasion *in vitro*.** *Oncogene* 1997, **15**:2049-2057.

53. Craig C, Wersto R, Kim M, Ohri E, Li Z, Katayose D, Lee SJ, Trepel J, Cowan K, Seth P: **A recombinant adenovirus expressing p27Kip1 induces cell cycle arrest and loss of cyclin-Cdk activity in human breast cancer cells.** *Oncogene* 1997, **14**:2283-2289.

54. Fueyo J, Gomez-Manzano C, Yung WK, Clayman GL, Liu TJ, Bruner J, Levin VA, Kyritsis AP: **Adenovirus-mediated p16/CDKN2 gene transfer induces growth arrest and modifies the transformed phenotype of glioma cells.** *Oncogene* 1996, **12**:103-110.

55. Frizelle SP, Grim J, Zhou J, Gupta P, Curiel DT, Geradts J, Kratzke RA: **Re-expression of p16INK4a in mesothelioma cells results in cell cycle arrest, cell death, tumor suppression and tumor regression.** *Oncogene* 1998, **16**:3087-3095.

56. Sandig V, Brand K, Herwig S, Lukas J, Barlek J, Strauss M: **Adenovirally transferred p16INK4/CDKN2 and p53 genes cooperate to induce apoptotic tumor cell death.** *Nat Med* 1997, **3**:313-319.

57. Chen J, Willingham T, Shuford M, Bruce D, Rushing E, Smith Y, Nisen PD: **Effects of ectopic overexpression of p21(WAF1/CIP1) on aneuploidy and the malignant phenotype of human brain tumor cells.** *Oncogene* 1996, **13**:1395-1403.

58. Eastham JA, Hall SJ, Sehgal I, Wang J, Timme TL, Yang G, Connell-Crowley L, Elledge SJ, Zhang WW, Harper JW: ***In vivo* gene therapy with p53 or p21 adenovirus for prostate cancer.** *Cancer Res* 1995, **55**:5151-5155.

59. Gotoh A, Kao C, Ko SC, Hamada K, Liu TJ, Chung LW: **Cytotoxic effects of recombinant adenovirus p53 and cell cycle regulator genes (p21 WAF1/CIP1 and p16CDKN4) in human prostate cancers.** *J Urol* 1997, **158**:636-641.

60. Joshi US, Dergham ST, Chen YQ, Dugan MC, Crissman JD, Vaitkevicius VK, Sartar FH: **Inhibition of pancreatic tumor cell growth in culture by p21WAF1 recombinant adenovirus.** *Pancreas* 1998, **16**:107-113.

61. Joshi US, Chen YQ, Kalemkerian GP, Adil MR, Kraut M, Sarkar FH: **Inhibition of tumor cell growth by p21WAF1 adenoviral gene transfer in lung cancer.** *Cancer Gene Ther* 1998, **5**:183-191.

62. Katayose D, Wersto R, Cowan KH, Seth P: **Effects of a recombinant adenovirus expressing WAF1/Cip1 on cell growth, cell cycle, and apoptosis.** *Cell Growth Differ* 1995, **6**:1207-1212.

63. Yang ZY, Perkins ND, Ohno T, Nabel EG, Nabel GJ: **The p21 cyclin-dependent kinase inhibitor suppresses tumorigenicity *in vivo* [see comments].** *Nat Med* 1995, 1:1052-1056.

64. Wang X, Gorospe M, Huang Y, Holbrook NJ: **p27Kip1 overexpression causes apoptotic death of mammalian cells.** *Oncogene* 1997, **15**:2991-2997.

65. Katayose Y, Kim M, Rakkar AN, Li Z, Cowan KH, Seth P: **Promoting apoptosis: a novel activity associated with the cyclin-dependent kinase inhibitor p27.** *Cancer Res* 1997, **57**:5441-5445.

66. McConnell BB, Starborg M, Brookes S, Peters G: **Inhibitors of cyclin-dependent kinases induce features of replicative senescence in early passage human diploid fibroblasts.** *Curr Biol* 1998, **8**:351-354.

67. Fahraeus R, Paramio JM, Ball KL, Lain S, Lane DP: **Inhibition of pRb phosphorylation and cell-cycle progression by a 20-residue peptide derived from p16CDKN2/INK4A.** *Curr Biol* 1996, **6**:84-91.

68. •• Fahraeus R, Lain S, Ball KL, Lane DP: **Characterization of the cyclin dependent kinase inhibitory domain of the INK4 family as a model for a synthetic tumour suppressor molecule.** *Oncogene* 1998, **16**:587-596.

The most recent paper on the CKI-based peptides that act as CDK inhibitors. These peptides have been linked to a 16 amino acid region of the antennapedia protein of *Drosophila*, which delivers the peptide inhibitors into live cells so that their cellular activity can be investigated. Interesting technology.

69. Kato D, Miyazawa K, Ruas M, Starborg M, Wada I, Oka T, Sakai T, Peters G, Hara E: **Features of replicative senescence induced by direct addition of antennapedia-p16INK4A fusion protein to human diploid fibroblasts.** *FEBS Lett* 1998, **427**:203-208.

70. Ball KL, Lain S, Fahraeus R, Smythe C, Lane DP: **Cell-cycle arrest and inhibition of Cdk4 activity by small peptides based on the carboxy-terminal domain of p21WAF1.** *Curr Biol* 1997, **7**:71-80.

71. Bonfanti M, Taverna S, Salmona M, D'Incalci M, Broggini M: **p21WAF1-derived peptides linked to an internalization peptide inhibit human cancer cell growth.** *Cancer Res* 1997, **57**:1442-1446.

72. Colas P, Cohen B, Jessen T, Grishina I, McCoy J, Brent R: **Genetic selection of peptide aptamers that recognize and inhibit cyclin-dependent kinase 2.** *Nature* 1996, **380**:548-550.

73. van den Heuvel S, Harlow E: **Distinct roles for cyclin-dependent kinases in cell cycle control.** *Science* 1993, **262**:2050-2054.

74. McPherson JP, Goldenberg GJ: **Induction of apoptosis by deregulated expression of DNA topoisomerase IIalpha.** *Cancer Res* 1998, **58**:4519-4524.

75. Meikrantz W, Schlegel R: **Suppression of apoptosis by dominant negative mutants of cyclin- dependent protein kinases.** *J Biol Chem* 1996, **271**:10205-10209.

76. Park DS, Levine B, Ferrari G, Greene LA: **Cyclin dependent kinase inhibitors and dominant negative cyclin dependent kinase 4 and 6**

promote survival of NGF-deprived sympathetic neurons. *J Neurosci* 1997, **17**:8975-8983.

77. Parker BW, Kaur G, Nieves-Neira W, Taimi M, Kohlhagen G, Shimizu T, Losiewicz MD, Pommier Y, Sausville EA, Senderowicz AM: **Early induction of apoptosis in hematopoietic cell lines after exposure to flavopiridol.** *Blood* 1998, **91**:458-465.

78. Schrump DS, Matthews W, Chen GA, Nixon A, Altorki NK: **Flavopiridol mediates cell cycle arrest and apoptosis in esophageal cancer cells.** *Clin Cancer Res* 1998, **4**:2885-2890.

79. Schutte B, Nieland L, Van Engeland M, Henfling ME, Meijer L, Ramaekers FC: **The effect of the cyclin-dependent kinase inhibitor olomoucine on cell cycle kinetics.** *Exp Cell Res* 1997, **236**:4-15.

80. Ongkeko W, Ferguson DJ, Harris AL, Norbury C: **Inactivation of Cdc2 increases the level of apoptosis induced by DNA damage.** *J Cell Sci* 1995, **108**:2897-2904.

81. Maas JW Jr, Horstmann S, Borasio GD, Anneser JM, Shooter EM, Kahle PJ: **Apoptosis of central and peripheral neurons can be prevented with cyclin-dependent kinase/mitogen-activated protein kinase inhibitors.** *J Neurochem* 1998, **70**:1401-1410.

82. Markus MA, Kahle PJ, Winkler A, Horstmann S, Anneser JM, Borasio GD: **Survival-promoting activity of inhibitors of cyclin-dependent kinases on primary neurons correlates with inhibition of c-Jun kinase-1.** *Neurobiol Dis* 1997, **4**:122-133.

83. Park DS, Morris EJ, Stefanis L, Troy CM, Shelanski ML, Geller HM, Greene LA: **Multiple pathways of neuronal death induced by DNA-damaging agents, NGF deprivation, and oxidative stress.** *J Neurosci* 1998, **18**:830-840.

84. Gervais JL, Seth P, Zhang H: **Cleavage of CDK inhibitor p21(Cip1/Waf1) by caspases is an early event during DNA damage-induced apoptosis.** *J Biol Chem* 1998, **273**:19207-19212.

85. Levkau B, Koyamam H, Raines EW, Clurman BE, Herren B, Orth K, Roberts JM, Ross R: **Cleavage of p21Cip1/Waf1 and p27Kip1 mediates apoptosis in endothelial cells through activation of Cdk2: role of a caspase cascade.** *Mol Cell* 1998, **1**:553-563.

86. Park JA, Kim KW, Kim SI, Lee SK: **Caspase 3 specifically cleaves p21WAF1/CIP1 in the earlier stage of apoptosis in SK-HEP-1 human hepatoma cells.** *Eur J Biochem* 1998, **257**:242-248.

87. Zhou BB, Li H, Yuan J, Kirschner MW: **Caspase-dependent activation of cyclin-dependent kinases during Fas-induced apoptosis in Jurkat cells.** *Proc Natl Acad Sci USA* 1998, **95**:6785-6790.

88. O'Connor PM, Fan S: **DNA damage checkpoints: implications for cancer therapy.** *Prog Cell Cycle Res* 1996, **2**:165-173.

89. O'Connor PM: **Mammalian G1 and G2 phase checkpoints.** *Cancer Surv* 1997, **29**:151-182.

90. Harper JW, Elledge SJ: **The role of Cdk7 in CAK function, a retro-retrospective.** *Genes Dev* 1998, **12**:285-289.

91. Yao SL, McKenna KA, Sharkis SJ, Bedi A: **Requirement of p34cdc2 kinase for apoptosis mediated by the Fas/APO-1 receptor and interleukin 1β-converting enzyme-related proteases.** *Cancer Res* 1996, **56**:4551-4555.

92. Wang Q, Fan S, Eastman A, Worland PJ, Sausville EA, O'Connor PM: **UCN-01: a potent abrogator of G2 checkpoint function in cancer cells with disrupted p53.** *J Natl Cancer Inst* 1996, **88**:956-965.

93. Poon RY, Chau MS, Yamashita K, Hunter T: **The role of Cdc2 feedback loop control in the DNA damage checkpoint in mammalian cells.** *Cancer Res* 1997, **57**:5168-5178.

94. Yu L, Orlandi L, Wang P, Orr MS, Senderowicz AM, Sausville EA, Silvestrini R, Watanabe N, Piwnica-Worms H, O'Connor PM: **UCN-01 abrogates G2 arrest through a Cdc2-dependent pathway that is associated with inactivation of the Wee1Hu kinase and activation of the Cdc25C phosphatase.** *J Biol Chem* 1998, **273**:33455-33464.

95. Jin P, Hardy S, Morgan DO: **Nuclear localization of cyclin B1 controls mitotic entry after DNA damage.** *J Cell Biol* 1998, **141**:875-885.

96. Jin P, Gu Y, Morgan DO: **Role of inhibitory CDC2 phosphorylation in radiation-induced G2 arrest in human cells.** *J Cell Biol* 1996, **134**:963-970.

97. Blasina A, Paegle ES, McGowan CH: **The role of inhibitory phosphorylation of CDC2 following DNA replication block and radiation-induced damage in human cells.** *Mol Biol Cell* 1997, **8**:1013-1023.

98. Arber N, Doki Y, Han EK, Sgambato A, Zhou P, Kim NH, Delohery T, Klein MG, Holt PR, Weinstein IB: **Antisense to cyclin D1 inhibits the growth and tumorigenicity of human colon cancer cells.** *Cancer Res* 1997, **57**:1569-1574.

99. Driscoll B, Wu L, Buckley S, Hall FL, Anderson KD, Warburton D: **Cyclin D1 antisense RNA destabilizes pRb and retards lung cancer cell growth.** *Am J Physiol* 1997, **273**:L941-949.

100. Kornmann M, Arber N, Korc M: **Inhibition of basal and mitogen-stimulated pancreatic cancer cell growth by cyclin D1 antisense is associated with loss of tumorigenicity and potentiation of cytotoxicity to cisplatinum.** *J Clin Invest* 1998, **101**:344-352.

101. Suzuki J, Isobe M, Morishita R, Aoki M, Horie S, Okubo Y, Kaneda Y, Sawa Y, Matsuda H, Ogihara T, Sekiguchi M: **Prevention of graft coronary arteriosclerosis by antisense cdk2 kinase oligonucleotide [see comments].** *Nat Med* 1997, **3**:900-903.

102. Hershko A: **Roles of ubiquitin-mediated proteolysis in cell cycle control.** *Curr Opin Cell Biol* 1997, **9**:788-799.

103. King RW, Deshaies RJ, Peters JM, Kirschner MW: **How proteolysis drives the cell cycle.** *Science* 1996, **274**:1652-1659.

104. Rolfe M, Chiu MI, Pagano M: **The ubiquitin-mediated proteolytic pathway as a therapeutic area.** *J Mol Med* 1997, **75**:5-17.

105. Coats S, Flanagan WM, Nourse J, Roberts JM: **Requirement of p27Kip1 for restriction point control of the fibroblast cell cycle.** *Science* 1996, **272**:877-880.

106. Wang S, Wuu J, Savas L, Patwardhan N, Khan A: **The role of cell cycle regulatory proteins, cyclin D1, cyclin E, and p27 in thyroid carcinogenesis.** *Hum Pathol* 1998, **29**:1304-1309.

107. Piva R, Cavalla P, Bortolotto S, Cordera S, Richiardi P, Schiffer D: **p27/kip1 expression in human astrocytic gliomas.** *Neurosci Lett* 1997, **234**:127-130.

108. Thomas GV, Szigeti K, Murphy M, Draetta G, Pagano M, Loda M: **Down-regulation of p27 is associated with development of colorectal adenocarcinoma metastases.** *Am J Pathol* 1998, **153**:681-687.

109. Guo Y, Sklar GN, Borkowski A, Kyprianou N: **Loss of the cyclin-dependent kinase inhibitor p27(Kip1) protein in human prostate cancer correlates with tumor grade.** *Clin Cancer Res* 1997, **3**:2269-2274.

110. Porter PL, Malone KE, Heagerty PJ, Alexander GM, Gatti LA, Firpo EJ, Darling JR, Roberts JM: **Expression of cell-cycle regulators p27Kip1 and cyclin E, alone and in combination, correlate with survival in young breast cancer patients.** *Nat Med* 1997, **3**:222-225.

111. Fero ML, Randel E, Gurley KE, Roberts JM, Kemp CJ: **The murine gene p27Kip1 is haplo-insufficient for tumour suppression.** *Nature* 1998, **396**:177-180.